

CLAIMS

1. A method for amplifying a target nucleic acid sequence comprising a target nucleic acid:
 - a) hybridizing a Riboprimer to a single stranded DNA template comprising the target nucleic acid sequence;
 - b) optionally hybridizing a blocking oligo to a region of the template which is 5' with respect to hybridization of the Riboprimer to the template;
 - c) extending the Riboprimer with a DNA polymerase; and
 - d) cleaving the annealed Riboprimer with an RNase H enzyme such that another Riboprimer hybridizes to the template and repeats primer extension by strand displacement, whereby multiple copies of the complementary sequence of the target sequence are produced.
2. The method of claim 1, wherein a plurality of Riboprimers is used.
3. The method of claim 1 wherein the Riboprimer comprises only ribonucleotides.
4. The method of claim 1 wherein the Riboprimer comprises at least one pyrimidine 2'-deoxyribonucleotide having a 2'-substituent on the sugar moiety.
5. The method of claim 1 wherein the Riboprimer comprises at least one pyrimidine 2'-fluoro-2'-deoxyribonucleotide.
6. The method of claim 1 wherein the Riboprimer comprises purine ribonucleotides and pyrimidine 2'-fluoro-2'-deoxyribonucleotides.
7. The method of claim 1 wherein the Riboprimer comprises AMP, GMP, 2'-F-dUMP and 2'-F-dCMP.
8. The method of claim 1 wherein the Riboprimer comprises at least one 2'-amino-2'-deoxyribonucleotide.

9. The method of claim 1 wherein the Riboprimer comprises at least one 2'-methoxy-2'-deoxyribonucleotide.
10. The method of claim 1 wherein the Riboprimer comprises at least one 2'-azido-2'-deoxyribonucleotide.
11. The method of claim 1 wherein the Riboprimer comprises at least one 2'-amino-2'-deoxyribonucleotide.
12. The method of claim 1 wherein the blocking oligo comprises a peptide nucleic acid (PNA).
13. The method of claim 1, wherein step (c) comprises at least one type of labeled dNTP such that labeled products are generated.
14. The method of claim 1, wherein the DNA polymerase is a thermostable DNA polymerase.
15. The method of claim 1, wherein the DNA polymerase is IsoTherm™ DNA polymerase.
16. The method of claim 1, wherein the DNA polymerase is SequiTherm™ DNA polymerase.
17. The method of claim 1, wherein the DNA polymerase is *Bst* DNA polymerase.
18. The method of claim 1, wherein the DNA polymerase is *Bca* DNA polymerase.
19. The method of claim 1, wherein the DNA polymerase is a non-thermostable DNA polymerase.
20. The method of claim 1, wherein the DNA polymerase is phi29 DNA polymerase.

21. The method of claim 20, wherein the DNA polymerase is RepliPHI™ DNA polymerase.
22. The method of claim 1, wherein the DNA polymerase is Exo-minus Klenow DNA polymerase.
23. The method of claim 1, wherein the RNase H enzyme is a thermostable RNase H.
24. The method of claim 23, wherein the thermostable RNase H is Hybridase™ thermostable RNase H.
25. The method of claim 1, wherein RNase H enzyme is Tth RNase H.
26. The method of claim 1, wherein the RNase H enzyme is Tfl RNase H.
27. The method of claim 1, wherein the RNase H enzyme is E.coli RNase H.
28. The method of claim 1, wherein steps (a) and (b) are performed in either order.
29. The method of claim 1, wherein steps (a) and (b) are performed simultaneously.
30. The method of claim 1, wherein steps (a) and (b) and (c) are performed simultaneously.
31. The method of claim 1, wherein steps (a) and (b) are performed before step (c).
32. The method of claim 1, wherein all steps are performed simultaneously.
33. A method of producing a microarray, comprising (i) amplifying a target nucleic acid sequence by the method of claim 1; and (ii) attaching the amplified products onto a solid substrate to make a microarray of the amplified products.

34. A method of producing a microarray, comprising (i) amplifying a target nucleic acid sequence by the method of claim 1; and (ii) hybridizing the amplified products to a microarray of nucleic acid molecules immobilized on a surface of a solid phase.

35. A composition comprising a complex of (a) a template strand; (b) a Riboprimer;

36. A kit for amplification of a target nucleic acid sequence, comprising a Riboprimer.

37. A method of generating multiple copies of a polynucleotide sequence complementary to an RNA sequence of interest, the method comprising the steps of:

- a) extending a first primer hybridized to a target RNA with an RNA-dependent DNA polymerase, wherein the first primer is a Riboprimer, whereby a complex comprising a first primer extension product and the target RNA is produced;
- b) cleaving RNA in the complex of step (a) with an RNase H enzyme;
- c) extending a second primer hybridized to the first primer extension product with a DNA-dependent DNA polymerase, whereby a second primer extension product is produced to form a complex of first and second primer extension products;
- d) cleaving the Riboprimer in the complex of first and second primer extension products with an RNase H enzyme such that a Riboprimer hybridizes to the second primer extension product; and
- e) extending the Riboprimer hybridized to the second primer extension product with a DNA-dependent DNA polymerase; whereby the first primer extension product is displaced, and whereby multiple copies of a polynucleotide sequence complementary to the RNA sequence of interest are generated.

38. The method of claim 37, wherein the RNA-dependent DNA polymerase is Bst DNA polymerase.

39. The method of claim 37, wherein the RNA-dependent DNA polymerase is IsoTherm™ DNA polymerase.

40. The method of claim 37, wherein the RNA-dependent DNA polymerase is Moloney murine leukemia virus (MMLV) reverse transcriptase.

41. The method of claim 40, wherein the MMLV reverse transcriptase is an RNase H minus MMLV reverse transcriptase.

42. The method of claim 37, wherein the RNA-dependent DNA polymerase is avian myeloblastosis virus (AMV) reverse transcriptase.

43. The method of claim 37, wherein a plurality of Riboprimers is used to generate multiple copies of a polynucleotide sequence complementary to the RNA sequence of interest.

44. The method of claim 37 wherein the Riboprimer comprises only ribonucleotides.

45. The method of claim 37 wherein the Riboprimer comprises at least one pyrimidine 2'-deoxyribonucleotide having a 2'-substituent on the sugar moiety.

46. The method of claim 37 wherein the Riboprimer comprises at least one pyrimidine 2'-fluoro-2'-deoxyribonucleotide.

47. The method of claim 37 wherein the Riboprimer comprises purine ribonucleotides and pyrimidine 2'-fluoro-2'-deoxyribonucleotides.

48. The method of claim 37 wherein the Riboprimer comprises AMP, GMP, 2'-F-dUMP and 2'-F-dCMP.

49. The method of claim 37 wherein the Riboprimer comprises at least one 2'-amino-2'-deoxyribonucleotide.

50. The method of claim 37 wherein the Riboprimer comprises at least one 2'-methoxy-2'-deoxyribonucleotide.

51. The method of claim 37 wherein the Riboprimer comprises at least one 2'-azido-2'-deoxyribonucleotide.

52. The method of claim 37 wherein the Riboprimer comprises at least one 2'-amino-2'-deoxyribonucleotide.

53. The method of claim 37, wherein the Riboprimer that hybridizes to the target RNA comprises a 5' portion that is not hybridizable to the target RNA under conditions under which the Riboprimer hybridizes to the target RNA.

54. The method of claim 37, wherein the Riboprimer that hybridizes to the target RNA comprises a poly-dU sequence.

55. The method of claim 54, wherein the target RNA is mRNA.

56. The method of claim 37, wherein the Riboprimer that hybridizes to the target RNA is a random primer.

57. The method of claim 37, wherein the target RNA is mRNA, and the Riboprimer that hybridizes to the target RNA comprises a poly-dU sequence, and further comprises a 5' portion that is not hybridizable to the target mRNA under conditions under which the Riboprimer hybridizes to the target RNA.

58. The method of claim 37, wherein a plurality of different Riboprimers are used for hybridizing to the target RNA.

59. The method of claim 37, wherein the RNase H enzyme is a thermostable RNase H.

60. The method of claim 59, wherein the thermostable RNase H is Hybridase™ thermostable RNase H.

61. The method of claim 37, wherein RNase H enzyme is Tth RNase H.

62. The method of claim 37, wherein the RNase H enzyme is Tfl RNase H.
63. The method of claim 37, wherein the RNase H enzyme is E.coli RNase H.
64. The method of claim 37, wherein the second primer comprises a fragment of the target RNA hybridized to the primer extension product, the fragment generated by cleaving RNA in the complex of step (b) with an enzyme that cleaves RNA from an RNA/DNA hybrid.
65. The method of claim 37, wherein the second primer comprises DNA.
66. The method of claim 37, wherein the second primer is a random primer.
67. The method of claim 37, wherein the DNA polymerase is a thermostable DNA polymerase.
68. The method of claim 37, wherein the DNA-dependent DNA polymerase is IsoTherm™ DNA polymerase.
69. The method of claim 37, wherein the DNA-dependent DNA polymerase is SequiTherm™ DNA polymerase.
70. The method of claim 37, wherein the DNA-dependent DNA polymerase is *Bst* DNA polymerase.
71. The method of claim 37, wherein the DNA-dependent DNA polymerase is *Bca* DNA polymerase.
72. The method of claim 37, wherein the DNA-dependent DNA polymerase is a non-thermostable DNA polymerase.
73. The method of claim 37, wherein the DNA-dependent DNA polymerase is phi29 DNA polymerase.

74. The method of claim 37, wherein the DNA-dependent DNA polymerase is RepliPHIT[™] DNA polymerase.

75. The method of claim 37, wherein the DNA-dependent DNA polymerase is Exo-minus Klenow DNA polymerase.

76. The method of claim 37, wherein the RNA-dependent DNA polymerase and DNA-dependent polymerase are the same enzyme.

77. The method of claim 37, wherein the RNA-dependent DNA polymerase and the enzyme that cleaves RNA from an RNA/DNA hybrid are the same enzyme.

78. The method of claim 37, wherein the DNA-dependent DNA polymerase and the enzyme that cleaves RNA from an RNA/DNA hybrid are the same enzyme.

79. The method of claim 37, wherein the DNA-dependent DNA polymerase, the RNA-dependent DNA polymerase and the enzyme that cleaves RNA from an RNA/DNA hybrid are the same enzyme.

80. The method of claim 37, wherein the method comprises generating multiple copies of a polynucleotide sequence complementary to two or more different sequences of interest.

81. The method of claim 80, wherein the method comprises at least two different Riboprimers that hybridize to the target RNA.

82. A method of producing a microarray, comprising (i) amplifying a polynucleotide sequence complementary to an RNA sequence of interest by the method of claim 37; and (ii) attaching the amplified products onto a solid substrate to make an microarray of the amplified products.

83. A method of producing a microarray, comprising (i) amplifying a polynucleotide sequence complementary to an RNA sequence of interest by the method of claim 37; and (ii) hybridizing the amplified products to a microarray of nucleic acid molecules immobilized on a surface of a solid phase.